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Mitochondrial dysfunction in neurodegenerative diseases

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Abstract

A potential pivotal role for mitochondrial dysfunction in neurodegenerative diseases is gaining increasing acceptance. Mitochondrial dysfunction leads to a number of deleterious consequences including impaired calcium buffering, generation of free radicals, activation of the mitochondrial permeability transition and secondary excitotoxicity. Neurodegenerative diseases of widely disparate genetic etiologies may share mitochondrial dysfunction as a final common pathway. Recent studies using cybrid cell lines suggest that sporadic Alzheimer's disease is associated with a deficiency of cytochrome oxidase. Friedreich's ataxia is caused by an expanded GAA repeat resulting in dysfunction of frataxin, a nuclear encoded mitochondrial protein involved in mitochondrial iron transport. This results in increased mitochondrial iron and oxidative damage. Familial amyotrophic lateral sclerosis is associated with point mutations in superoxide dismutase, which may lead to increased generation of free radicals and thereby contribute to mitochondrial dysfunction. Huntington's disease (HD) is caused by an expanded CAG repeat in an unknown protein termed huntingtin. The means by which this leads to energy impairment is unclear, however studies in both HD patients and a transgenic mouse model show evidence of bioenergetic defects. Mitochondrial dysfunction leads to oxidative damage which is well documented in several neurodegenerative diseases. Therapeutic approaches include methods to buffer intracellular ATP and to scavenge free radicals.

Keywords: Mitochondrion; Huntington's disease; Friedreich's ataxia; Alzheimer's disease; Amyotrophic lateral sclerosis; Oxidative damage

1. Introduction

Neurodegenerative diseases are a heterogeneous group of illnesses with distinct clinical phenotypes and genetic etiologies. Major advances in understanding the pathogenesis of these illnesses have come from molecular genetics. Despite the presence of genetic defects in widely varying proteins substantial evidence points to mitochondrial dysfunction as a unifying fundamental mechanism involved in neuronal degeneration. Mitochondrial dysfunction has widespread deleterious ramifications for cellular function which are discussed in greater detail in other subsections of this review. In brief mitochondrial dysfunction leads to impaired energy production, impaired cellular calcium buffering, activation of proteases and phospholipases, activation of nitric oxide synthase, and generation of free radicals. The above pathways can lead to either apoptotic or necrotic cell death depending on the severity of the insult.

The present review will focus on four prototypical neurodegenerative diseases which are associated with

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either nuclear or mitochondrial DNA mutations which either directly or indirectly lead to mitochondrial dysfunction. Recent evidence implicates a cytochrome oxidase deficiency in Alzheimer's disease. There is a mutation in a nuclear encoded mitochondrial protein in Friedreich's ataxia. In familial amyotrophic lateral sclerosis mitochondrial dysfunction may be a consequence of oxidative damage due to point mutations in Cu,Zn superoxide dismutase. In Huntington's disease the means by which the nuclear DNA encoded expansion of CAG repeats in huntingtin results in mitochondrial dysfunction remain to be clarified, yet increasing evidence implicates an impairment of bioenergetics in Huntington pathogenesis.

2. Alzheimer's disease

Alzheimer's disease (AD) is the most common of the neurodegenerative diseases. The most important risk factor is advancing age [1]. The illness occurs in both a familial form which is autosomal dominant inherited and an apparently sporadic illness. Familial autosomal dominant Alzheimer's disease is associated with point mutations in the amyloid precursor protein as well as in novel proteins entitled presenilin-1 and presenilin-2. Familial Alzheimer's disease accounts for approximately 5% of all cases. The remaining apparently sporadic cases of Alzheimer's disease show an increased risk in families of 2.5–3-fold. A sporadic inheritance pattern with familial association and evidence for maternal transmission are characteristic features of known mitochondrial genetic diseases. Some evidence has suggested that there is maternal inheritance in Alzheimer's disease [2,3]. These studies showed an increase in female to male ratio in the parental generation of Alzheimer's disease probands. In a group of families in which there were both an affected parent and at least two affected siblings the ratio of mothers to fathers in the parental generation was 9:1 [2].

There is substantial evidence implicating metabolic defects in Alzheimer's disease. Studies utilizing positron emission tomography consistently show reduced glucose metabolism in temporoparietal regions of Alzheimer's disease patients, and this appears to occur quite early in the disease course [4]. Recent studies have demonstrated that this occurs in patients at risk for Alzheimer's disease [5], and there appeared to be reduced glucose utilization in asymptomatic patients who are homozygous for the Apo ε4 allele, a known risk factor for sporadic Alzheimer's disease [6]. Positron emission tomography studies also show increased oxygen utilization in comparison with glucose utilization in Alzheimer's disease patients [7]. This latter observation has been confirmed with direct measurements in arterial and jugular venous samples [8,9]. Prior work also demonstrated abnormal glucose metabolism in brain biopsy specimens [10]. Phosphorus magnetic resonance spectroscopy has demonstrated abnormalities in either phosphocreatine (PCr) or inorganic phosphate (Pi) in Alzheimer's disease patients as compared with elderly controls [11,12]. The study of Smith and colleagues demonstrated a reduction in PCr/Pi ratio in the frontal cortex of Alzheimer's disease patients [13].

Initial studies which suggested that there were defects in cytochrome oxidase in Alzheimer's disease were done in platelets. Parker and colleagues reported significant decreases in cytochrome oxidase activity in Alzheimer's disease platelets as compared to normal controls [14]. This work was disputed utilizing less purified platelets preparations but was confirmed in a follow-up study [15,16]. Studies of postmortem cerebral tissue of Alzheimer's disease patients confirmed reduced cytochrome oxidase activity [17,18]. The cytochrome oxidase activity shows a reduction in catalytic activity yet normal amounts of cytochrome aa_3, suggesting that reduced complex IV activity is a consequence of abnormal catalytic activity rather than decreased enzyme levels [16].

Although prior biochemical studies suggested that there were decreases in cytochrome oxidase activity in Alzheimer's disease platelets and cerebral tissue, it was unclear whether this was a primary or secondary effect of the disease process. A novel technique to investigate the role mitochondrial defects is to utilize cybrid technology, which was pioneered by King and Attardi [19]. This technique involves the transfer of mitochondria from living patients or cell lines to mitochondria deficient cells (ρ^0 cells). Cell lines from a variety of sources can be depleted of mitochondrial DNA (mtDNA) by exposing them to low concentrations of ethidium bromide. Ethidium bromide is concentrated within mitochondria and preferentially in-

hibits mtDNA replication in comparison to nuclear DNA replication. Exposed cells lose their mtDNA and assume an anaerobic phenotype. Cybrids are cells formed by fusing mitochondria from platelets or other tissues into the ρ^0 cells. Since the ρ^0 cells are auxotrophic for uridine and pyruvate, any cells which are not transformed are then eliminated by removing uridine and pyruvate from the medium. The resulting cybrids then enable one to determine whether any observed defects in oxidative phosphorylation are attributable to alterations in the patient's mtDNA, since the patient's mitochondria now function in the presence of a different nuclear background.

Recent studies using the cybrid technique to demonstrated that the cytochrome oxidase defects in Alzheimer's disease appear to be encoded on mtDNA [20,21]. It was shown that cytochrome oxidase defects can be transferred from Alzheimer's disease platelets into cybrids. Furthermore the ensuing cybrid cell lines show markedly increased free radical production. Point mutations were found in the cytochrome oxidase-1 and cytochrome oxidase-2 mtDNA encoded subunits of cytochrome oxidase, however further work needs to be done to exclude the possibility that these mutations are not present in nuclear pseudogenes. Nuclear pseudogenes are mitochondrial DNA sequences which are randomly incorporated into the nuclear genome by unclear mechanisms, but which exist for much of the mitochondrial genome.

The consequences of cytochrome oxidase defects in cybrid cell lines on intracellular calcium buffering have been determined [22]. The Alzheimer's disease cybrids show elevated basal cytosolic calcium concentrations as well as enhanced sensitivity to inositol-1,4,5-triphosphate mediated calcium release. They also show slower recovery from the increased calcium levels. These findings are consistent with prior observations in AD fibroblasts [23–25]. They are also consistent with the finding of decreased calcium uptake in mitochondria from AD fibroblasts [26]. Impaired calcium buffering is also known to occur in fibroblasts of patients with a known mitochondrial disorder, MELAS syndrome [27].

Alzheimer's disease cybrid cell lines are associated with increased free radical production. One therefore might expect that there would be evidence for increased free radical damage in Alzheimer's disease postmortem tissue. Mitochondrial DNA may be preferentially vulnerable since it is located close to the inner mitochondrial membrane. Consistent with this possibility we found a 3-fold increase in 8-hydroxy-2-deoxyguanosine content of mtDNA in AD postmortem tissue as compared to age-matched controls [28]. In other studies there have been reports of increased tissue concentrations of malondialdehyde and protein carbonyl groups [29,30]. Furthermore novel spin trapping techniques demonstrated increased oxidative damage to both lipids and proteins [31]. Immunocytochemical studies have demonstrated that there is evidence for oxidative damage at the cellular level. Neurofibrillary tangle bearing neurons showed increased immunostaining with antibodies to advanced glycation end products, hemeoxygenase-1, malondialdehyde, 4-hydroxynonenal, protein carbonyl groups, carbonylated neurofilaments, and 3-nitrotyrosine [13,32–38]. Interestingly these antibodies show increased staining in cell bodies and neurites rather than in senile plaques, suggesting an intracellular source of free radicals.

Mitochondrial dysfunction may be linked to the other neuropathological hallmarks of Alzheimer's disease including senile plaques and neurofibrillary tangles. Previous studies showed that impairment of cytochrome oxidase in vitro leads to an increase in C-terminal fragments of the amyloid precursor protein, which contain the β-amyloid peptide [39], and a decrease in non-amyloidogenic processing of the amyloid precursor protein [40]. An increase in intracellular β-amyloid 1–42 was found after exposure of cultured guinea pig neurons to hydrogen peroxide, and oxidative stress increased β-amyloid in mammalian lens tissue [41,42]. An increase in intracellular β-amyloid was also observed in cultured astrocytes from Down syndrome patients in which there is increased free radical production [43]. Oxidative damage may lead to cross-linking and impaired solubility of β-amyloid [44,45].

Oxidative injury has also been shown to lead to intermolecular cross links in covalent bonds which could contribute to the generation of paired helical filaments [46]. Oxidation of critical cysteine residues seems to be associated with the aggregation of tau proteins into paired helical filaments [47]. Reduced ATP generation also leads to activation of ERK1

and ERK2 kinases which phosphorylate tau proteins into a paired helical filament-like state similar to that in AD [48,49].

3. Friedreich's ataxia

A role of mitochondrial dysfunction in Friedreich's ataxia has been greatly strengthened by recent observations. Friedreich's ataxia is characterized by neurodegeneration involving the spinocerebellar pathways as well as a cardiomyopathy. The gene product was recently cloned and designated frataxin [50]. Frataxin deficiency may be a consequence of nonsense or missense point mutations, but the primary cause appears to be an expansion of a polymorphic GAA trinucleotide repeat situated in the first intron of the corresponding gene. This results in marked reduction in steady state levels of mature frataxin mRNA. There appears to be a correlation between the regions of degeneration observed in the disease and the sites of frataxin transcription, which are highest in the heart, spinal cord and dorsal root ganglia [51]. Recent studies show that a gene from yeast is homologous to the human frataxin protein [52]. This gene encodes a mitochondrial protein involved in iron homeostasis and respiration function. Human frataxin has been linked to green fluorescent protein and was shown to be localized to mitochondria [51–54]. Disruption of the yeast homologous gene results in respiratory insufficiency with an inability to carry out oxidative phosphorylation [51,52,54], as well as a loss of mitochondrial DNA [52,54]. Yeast with disruption of their frataxin homologue show marked increases in iron transport, increases in iron content, and hypersensitivity to oxidative stress mediated by H_2O_2 [52]. These observations suggest that impaired function of this protein most likely leads to mitochondrial dysfunction and results in hypersensitivity to oxidative stress, presumably mediated by iron catalyzed Fenton chemistry. This speculation is supported by studies of endomyocardial biopsies of patients with Friedreich's ataxia [55]. The biopsies show deficiencies of aconitase and complexes I, II and III of the electron transport chains which contain iron-sulfur clusters and are known to be susceptible to oxidative stress. It is also of interest that a Friedreich's ataxia-like syndrome with retinitis pigmentosis is caused by mutations in the α-tocopherol transfer protein [56–58]. This results in reduced concentrations of vitamin E. Neurological symptoms in this disorder include ataxia, dysarthria, hyporeflexia and decreased proprioceptive sensation.

Other evidence implicating mitochondrial dysfunction in Friedreich's ataxia includes an increased incidence of diabetes mellitus and optic atrophy which are frequent complications of mitochondrial disorders. Positron emission tomography studies of Friedreich's ataxia patients have detected cerebral glucose hypermetabolism early in disease progression [59]. Biochemical studies found increased blood lactate levels and generally abnormal carbohydrate metabolism in patients with Friedreich's ataxia [60]. Reduced activities of several mitochondrial enzymes including α-ketoglutarate dehydrogenase and pyruvate dehydrogenase have also been reported [61].

4. Amyotrophic lateral sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS) is a prototypical neurodegenerative disease of late life characterized by progressive muscle weakness, atrophy and spasticity [62]. It leads to paralysis and death within 3–5 years after onset. Characteristic neuropathologic features are loss of anterior horn motor neurons as well as degeneration of the corticospinal tracts. Ninety percent of ALS cases are apparently sporadic with no identifiable genetic or environmental risk factors. The remaining 10% of cases show familial autosomal dominant inheritance. A major advance in the understanding of ALS was the identification of point mutations in the enzyme superoxide dismutase in approximately 25% of patients who have familial ALS [63]. More than 60 mutations have now been associated with the disease. These mutations typically affect the protein backbone of the enzyme and may interfere with normal dimer interactions. Only two mutations have thus far been reported which may affect the active site copper.

The observation that mutations in superoxide dismutase cause familial ALS suggested that oxidative injury might be playing role in its pathogenesis. Although there is a decrease in superoxide dismutase activity in familial ALS patients, strong evidence sug-

gests that the genetic defect leads to a gain of function from the mutant enzyme. This evidence includes the dominant inheritance pattern of familial ALS, the lack of correlation between enzyme activity and disease severity [64], and the observation that overexpression of the mutant enzyme in transgenic mice leads to motor neuron degeneration [65–67]. The mutations appear to alter the stability of the protein backbone and reduce the half-life of the molecule [68]. They also appear to alter zinc binding [69]. These changes may relax the conformation of the active channel thereby allowing increased access of hydrogen peroxide or peroxynitrite to the active site copper. This is predicted to increase generation of hydroxyl radicals or nitronium ions which can then nitrate proteins [70]. In support of this two in vitro studies showed that superoxide dismutase with two different familial ALS mutations can generate increased amounts of hydroxyl radicals as compared to wild-type molecules [71–73]. Furthermore we recently obtained evidence in two different strains of transgenic ALS mice that there are increases in 3-nitrotyrosine concentrations, consistent with increased accessibility of peroxynitrite to the active site copper [74,75]. Of interest in both of these lines of transgenic ALS mice mitochondrial vacuolization and swelling are prominent pathologic features [65,67]. Furthermore we found increased complex I activity in both postmortem brain material of patients with the A4V superoxide dismutase mutation as well as in transgenic mice with the G93A superoxide dismutase mutation [76]. It is possible that increased generation of free radicals may damage the inner mitochondrial membrane. This could lead to a proton leak which would therefore have to be compensated by increased activity of the mitochondrial electron transport complexes responsible for proton transport. Expression of superoxide dismutase with the G93A mutation in neuroblastoma cells in vitro leads to a loss of mitochondrial membrane potential and increases in cytosolic calcium [77].

In sporadic ALS there is also evidence to suggest impairment of energy metabolism. Reduced glucose metabolism has been observed in cerebral cortex as sporadic ALS patients using positron emission tomography [78,79]. We recently examined electron transport enzymes in postmortem tissue of sporadic ALS patients. No alterations were found but this could be due to heterogeneity of defects amongst patients, which would obscure them due to an averaging effect [76]. Furthermore abnormalities could be obscured by astrogliosis. A recent study using the cybrid cell technique showed that there appeared to be mild decreases in complex I and complex IV activities associated with sporadic ALS [80]. Furthermore peripheral blood lymphocytes from sporadic ALS patients show increased cytosolic calcium and impaired responses to uncouplers of oxidative phosphorylation [81]. Studies of motor neurons in sporadic ALS patients show an accumulation of mitochondria in proximal axons, and muscle biopsies show increased mitochondrial volume and calcium levels [82–84]. Liver biopsies show enlarged mitochondria with intramitochondrial inclusions in sporadic ALS patients [85]. Mitochondria with abnormal protrusions were observed in anterior horn cells of a familial ALS patient who was later determined to have a superoxide dismutase mutation [86].

A consequence of mitochondrial dysfunction in either familial or sporadic ALS patients may be increased oxidative damage. Both we and others have observed increased protein carbonyl groups in the motor cortex and spinal cord of ALS patients [76,87]. We recently found increased concentrations of 3-nitrotyrosine and its major metabolite 3-nitro-4-hydroxyphenylacetic acid in the thoracic and lumbar spinal cord of both sporadic and familial ALS patients [88]. Immunocytochemical staining also demonstrated increased 3-nitrotyrosine in anterior horn cells.

A pathologic hallmark of familial and sporadic ALS is the accumulation of neurofilaments in proximal axons [89,90]. This may be a consequence of oxidative damage or it could be due to accumulation of 3-nitrotyrosine in the neurofilament light chain [70], which impairs normal aggregation of neurofilaments [91,92]. Furthermore an energy defect may contribute to slowing to axonal transport which has recently been demonstrated in sporadic ALS patients, in which there was an accumulation of mitochondria in proximal axons [83].

5. Huntington's disease

The genetic defect in Huntington's disease (HD)

consists of an expanded CAG repeat in a gene located on chromosome 4. The gene encodes a novel protein designated huntingtin which is widely distributed in both the peripheral tissues of the body as well as in the central nervous system. Both the normal function of huntingtin as well as its role in the pathogenesis of neuronal degeneration in Huntington's disease are obscure. Substantial evidence however suggests that the disease mutation leads to a gain of function causing cell type specific neuronal degeneration predominantly in the striatum. Evidence in support of this includes the observation that heterozygote knockout mice of the gene huntingtin do not show any phenotypic abnormalities [93]. The cleavage of huntingtin leads to N-terminal fragments which aggregate to form intranuclear inclusion bodies and aggregates in dystrophic neurites [94].

There is a substantial body of evidence implicating defects in energy metabolism in HD. Decreased glucose metabolism using positron emission tomography has been consistently shown in HD patients. The reductions in glucose metabolism occur in presymptomatic patients [29]. Furthermore we utilized proton magnetic resonance spectroscopy to demonstrate that there are elevated lactate concentrations in both the occipital cortex and in the basal ganglia of HD patients [95]. Increased lactate concentrations occur in the basal ganglia of some but not all presymptomatic patients, suggesting that the metabolic defects can precede clinical manifestations of the illness. Other authors found increased lactate in both frontal cortex as well as in occipital cortex and cerebellum [96,97]. We observed increased lactate concentrations in both parietal and supplementary motor cortex, suggesting that there is a widespread metabolic defect in Huntington's disease brain tissue. Furthermore we found a decrease in PCr/Pi ratio in resting gastrocnemius muscle of HD patients, providing evidence that there are defects in energy metabolism in peripheral tissues as well [98]. The latter observation is consistent with reports of progressive weight loss in HD patients despite increased caloric intake [99]. Furthermore we found that cerebrospinal fluid lactate to pyruvate ratios are increased in HD subjects as compared to age-matched controls, and similar observations have been made in patients with Machado-Joseph disease which is also due to an expansion of CAG repeats [98,100].

Reports of changes in enzymes involved in oxidative phosphorylation have shown reduced succinate dehydrogenase activity in HD postmortem brain tissue [101,102]. Reports of mitochondrial electron transport enzymes yielded inconsistent results. Two recent studies however demonstrated a 55–60% decrease in complex II–III activity in Huntington's disease basal ganglia [103,104]. Smaller decreases in cytochrome oxidase activity were less consistent. Complex I and citrate synthase activity showed no changes. The most consistent alterations in electron transport activity therefore appear to be decreases in complex II–III activity in the basal ganglia of HD patients. This is of interest since genetic defects affecting complex II–III activity are associated with basal ganglia degeneration [105], and comparable defects in experimental animals result in striatal degeneration [106]. Studies in cultured fibroblasts from HD and control patients also show mitochondrial defects [107]. Ionomycin induced calcium influxes result in depolarization of the mitochondrial membrane potential. Normal fibroblasts show depolarization followed by recovery, but Huntington's disease fibroblasts show failure to recovery fully after the second application of ionomycin.

One proposed mechanism by which the HD gene defect could lead to impaired energy metabolism is by an interaction between huntingtin and glyceraldehyde-3-phosphate dehydrogenase, a critical component of the glycolytic pathway [108]. It was suggested that an increase in polyglutamine repeats in huntingtin potentially inhibits the enzyme. Indeed we found that intrastriatal administration of the glyceraldehyde-3-phosphate dehydrogenase inhibitor iodoacetate produces dose dependent striatal lesions, which correlate with inhibition of enzyme activity [109]. However we could not confirm a decrease in glyceraldehyde-3-phosphate dehydrogenase activity in postmortem brain tissue of Huntington's disease patients [104], however a competitive inhibition could still occur. There is limited evidence for oxidative damage postmortem brain tissue of Huntington's disease patients. We found increased concentrations of 8-hydroxy-2-deoxyguanosine in nuclear DNA in Huntington's disease caudate [104]. Furthermore our initial immunocytochemical studies showed increased staining of striatal neurons of HD patients with antibodies to hemeoxygenase-1, 8-hydroxy-2-de-

oxyguanosine and malondialdehyde modified protein [110].

Other evidence implicating mitochondrial dysfunction in the pathogenesis of Huntington's disease comes from studies of mitochondrial toxins. We utilized 3-nitropropionic acid, an irreversible inhibitor of succinate dehydrogenase, to attempt to model Huntington's disease in both rodents and primates [106,111]. Accidental ingestion of 3-nitropropionic acid in man produces selective basal ganglia lesions and dystonia [112]. Systemic administration of 3-nitropropionic acid to nonhuman primates results in both a movement disorder as well as frontal type cognitive deficits which are similar to those which occur in HD [106,113]. Histological evaluation showed basal ganglia degeneration with sparing of NADPH diaphorase interneurons, dendritic abnormalities in spiny neurons and sparing of the nucleus accumbens, all of which are characteristic features of Huntington's disease neuropathology.

Further evidence for a metabolic defect in HD comes from a recent transgenic animal model [114]. Transgenic mice were made expressing a large number of CAG repeats (130–140) in exon 1 of the HD gene. These mice developed normally for about 8 weeks followed by onset of a movement disorder with tremors and subsequent seizures. The mice die at approximately 12–14 weeks of age. Intranuclear inclusion bodies which stain with N-terminal antibodies to huntingtin develop preceding the onset of symptoms [20]. It has therefore been suggested that these nuclear inclusion bodies may play a role in disease pathogenesis. Although there is no degeneration of the basal ganglia the brains appear to be small, and this occurs early in the disease process.

A characteristic feature of these mice is progressive weight loss despite increased caloric intake [114]. These findings are consistent with those observed in Huntington's disease patients who also appear to have increased caloric intake despite weight loss [99]. These findings therefore suggest that there may indeed be a metabolic defect in both a transgenic animal model of HD as well as in patients. Furthermore we have recently studied another transgenic animal model of HD in which there is a expansion of either 48 or 66 repeats in the full length huntingtin protein (Browne et al., unpublished findings). These mice as yet have not shown any phenotypic abnormalities. The mice however show increased 2-deoxyglucose uptake in the basal ganglia, further suggesting a metabolic defect associated with the CAG expansion in the huntingtin protein.

6. Therapeutic strategies

If a defect in energy metabolism underlies the pathogenesis of neurodegenerative diseases then a reasonable therapeutic strategy is to utilize compounds which improve mitochondrial function. Coenzyme Q_{10} or ubiquinone is an essential component of the electron transport chain where it serves as an electron donor and acceptor. It is also a potent antioxidant, particularly in mitochondria. Prior work demonstrated that it protects against glutamate toxicity in cultured cerebellar neurons [115]. We demonstrated that it produces dose-dependent protection against striatal lesions produced by the succinate dehydrogenase inhibitor malonate [111]. More recently we found that it produces marked neuroprotection against 3-nitropropionic acid toxicity, and also improves survival in a transgenic animal model of ALS associated with point mutations and superoxide dismutase (Beal et al., unpublished data). It protects against malonate ATP depletions [111], and it protects against MPTP induced dopamine depletions in older mice [116]. Examination of its effects in HD patients show that it produced a significant 36% decrease in occipital cortex lactate concentrations as assessed by magnetic resonance spectroscopy [98]. Following withdrawal of the coenzyme Q_{10} treatment the lactate levels return to baseline. It produced additive effects with the *N*-methyl-D-aspartate (NMDA) antagonist MK-801 against malonate toxicity in vivo [117]. In view of these neuroprotective effects a clinical trial has been designed to assess the effects of coenzyme Q_{10} with or without an NMDA antagonist in the treatment of Huntington's disease patients.

Another novel therapeutic strategy to ameliorate mitochondrial induced dysfunction is to attempt to buffer intracellular energy stores. The major energy source in the brain is ATP which is tightly coupled to phosphocreatine. A therapeutic strategy may therefore be to administer creatine to attempt to increase phosphocreatine and ATP concentrations within the brain. Creatine kinase catalyzes the reaction of ATP

with phosphocreatine to generate ATP [118]. ATP generated by oxidative phosphorylation is transported through the mitochondrial inner membrane by the adenine nucleotide transporter, where it is transphosphorylated with creatine by the mitochondrial creatine kinase to generate phosphocreatine. Phosphocreatine then leaves the mitochondria and diffuses to the cytoplasm where it serves as both a temporal and spatial energy buffer. Phosphocreatine maintains ATP levels utilized by the sodium potassium ATPase and the calcium ATPase [119]. It serves to maintain membrane potential and to restore ion gradients after neurotransmitter release, consistent with the localization of high amounts of creatine kinase to brain regions rich in synaptic connections [120]. Its importance to brain function is supported by in vivo ^{31}P NMR transfer measurements showing a correlation of creatine kinase flux with brain activity measured by the EEG as well as with brain 2-deoxyglucose uptake [121,122].

Oral administration of creatine stimulates mitochondrial respiration and phosphocreatine synthesis which may help sustain ATP levels under stress conditions [123]. Furthermore phosphocreatine serves as a direct energy source for glutamate uptake into synaptic vesicles [124]. We found that administration of creatine in the diet produced significant protection against both malonate and 3-nitropropionic acid induced neurotoxicity [125]. Creatine increased brain levels of phosphocreatine and ATP and protected against 3-nitropropionic acid induced depletions. Creatine also protected against 3-nitropropionic acid induced increases in striatal lactate concentrations as assessed by proton magnetic resonance spectroscopy. Furthermore creatine administration protected against malonate induced increases in hydroxyl radical generation and increases in 3-nitrotyrosine, and 3-nitropropionic acid induced increases in 3-nitrotyrosine, which may be a downstream consequence of energy impairment. These observations suggest that creatine administration may be a novel therapeutic strategy for the treatment of neurodegenerative diseases.

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